```
HU:MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSL
MO:MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSL


HU:GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTVHFSGQFTGTAG
MO:GGPAPPPAPPPPPPPPPHSFIKQEPSWGGAEPHEEQCLSAFTLHFSGQFTGTAG


HU:ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYSTVTFDGTPS
MO:ACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYSTVTFDGAPS


HU:YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG
MO:YGHTPSHHAAQFPNHSFKHEDPMGQQGSLGEQQYSVPPPVYGCHTPTDSCTG


HU:SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTE
MO:SQALLLRTPYSSDNLYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTE


HU:GQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDVRRVPGVAPTLVRSAS
MO:GQSNHGIGYESDNETAPILCGAQYRIHTHGVFRGIQDVRRVSGVAPTLVRSAS


HU:ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR
MO:ETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSR


HU:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR
MO:SDQLKRHQRRHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCR


HU:WPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
MO:WHSCQKKFARSDELVRHHNMHQRNMTKLHVAL
```

FIG. 1

52 kD -   - 52 kD

1 2 3 4

FIG. 2

52 kD -   - 52 kD

1   2   3   4   5   6

control antibody   non immunized   immunized
B6 sera   B6 sera

FIG. 3

52 kD - ⟍   ⟍ 52 kD

1   2   3   4   5   6

control antibody   non immunized   immunized
                        B6 sera   B6 sera
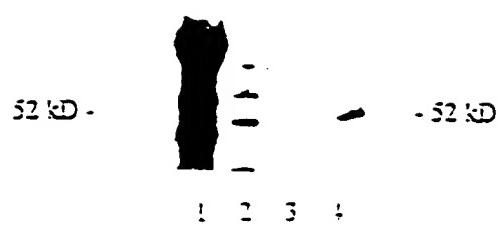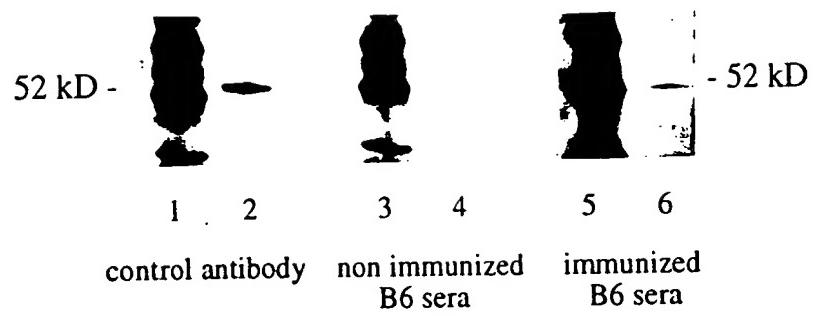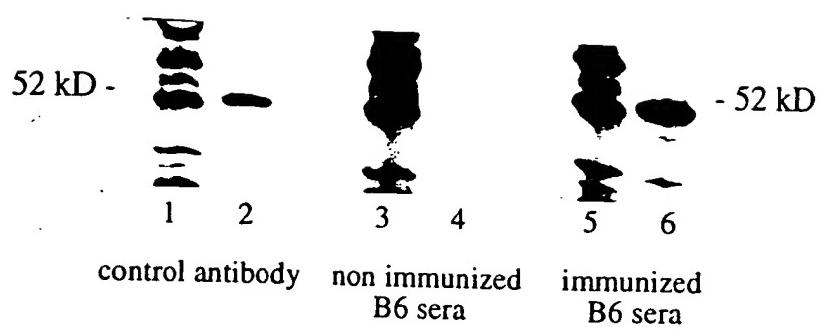
FIG. 4
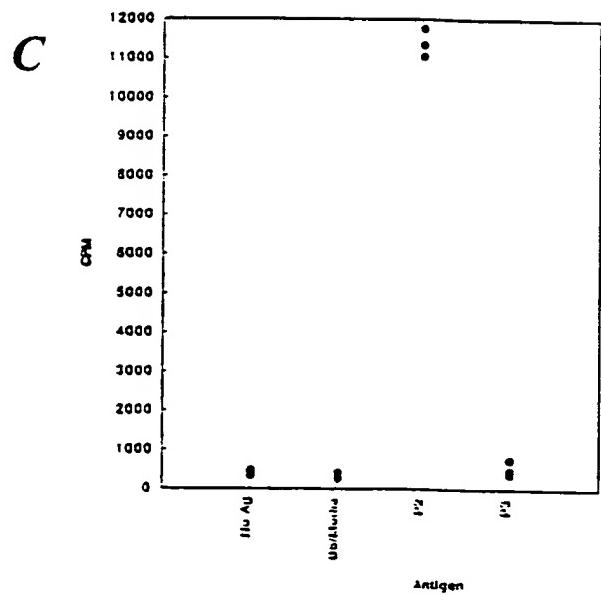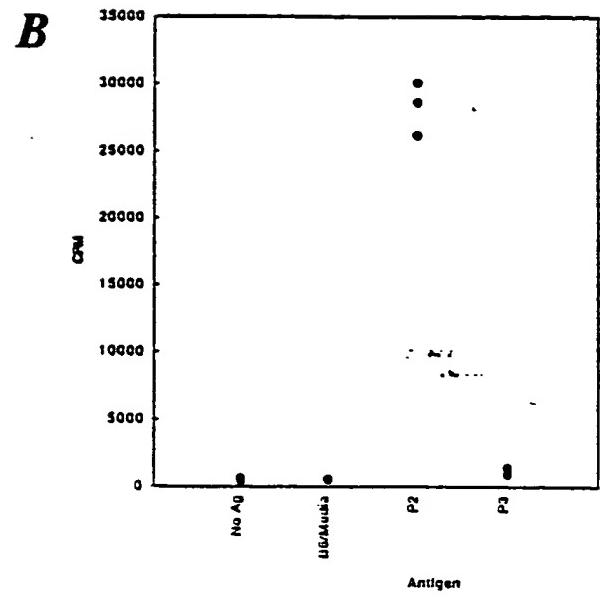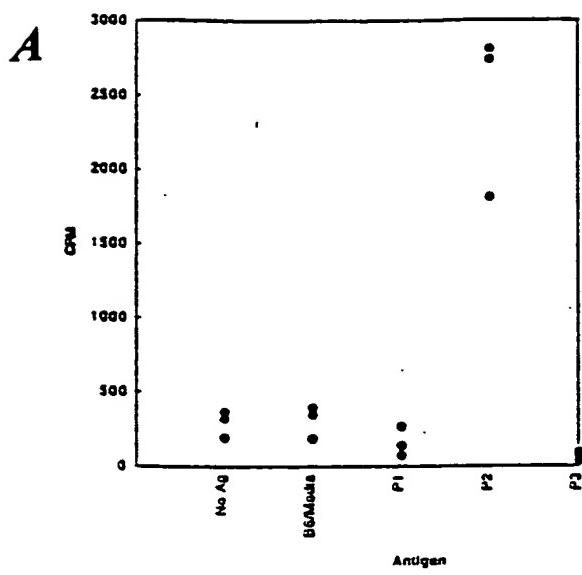
FIG. 5A-5C

A   **Vaccine A stimulated line**   **B**   **Vaccine B stimulated line**
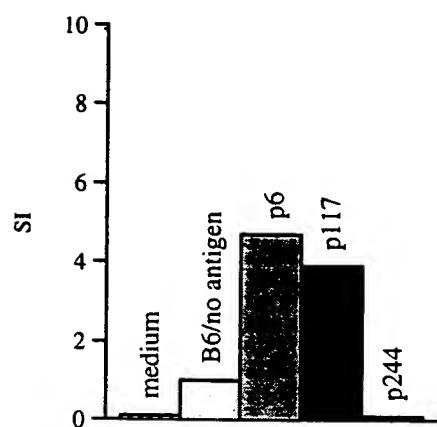
FIG. 6A and 6B

A

p117-139 stimulated line

B

p117-139 stimulated clone

C

p6-22 stimulated line

D

p6-22 stimulated clone
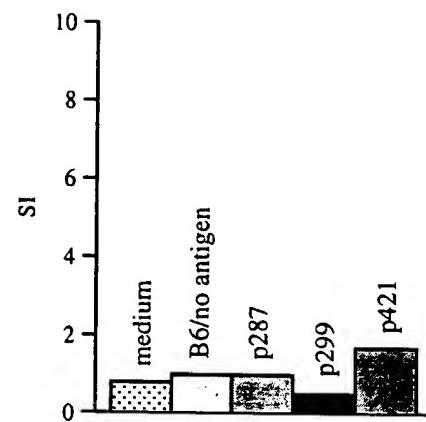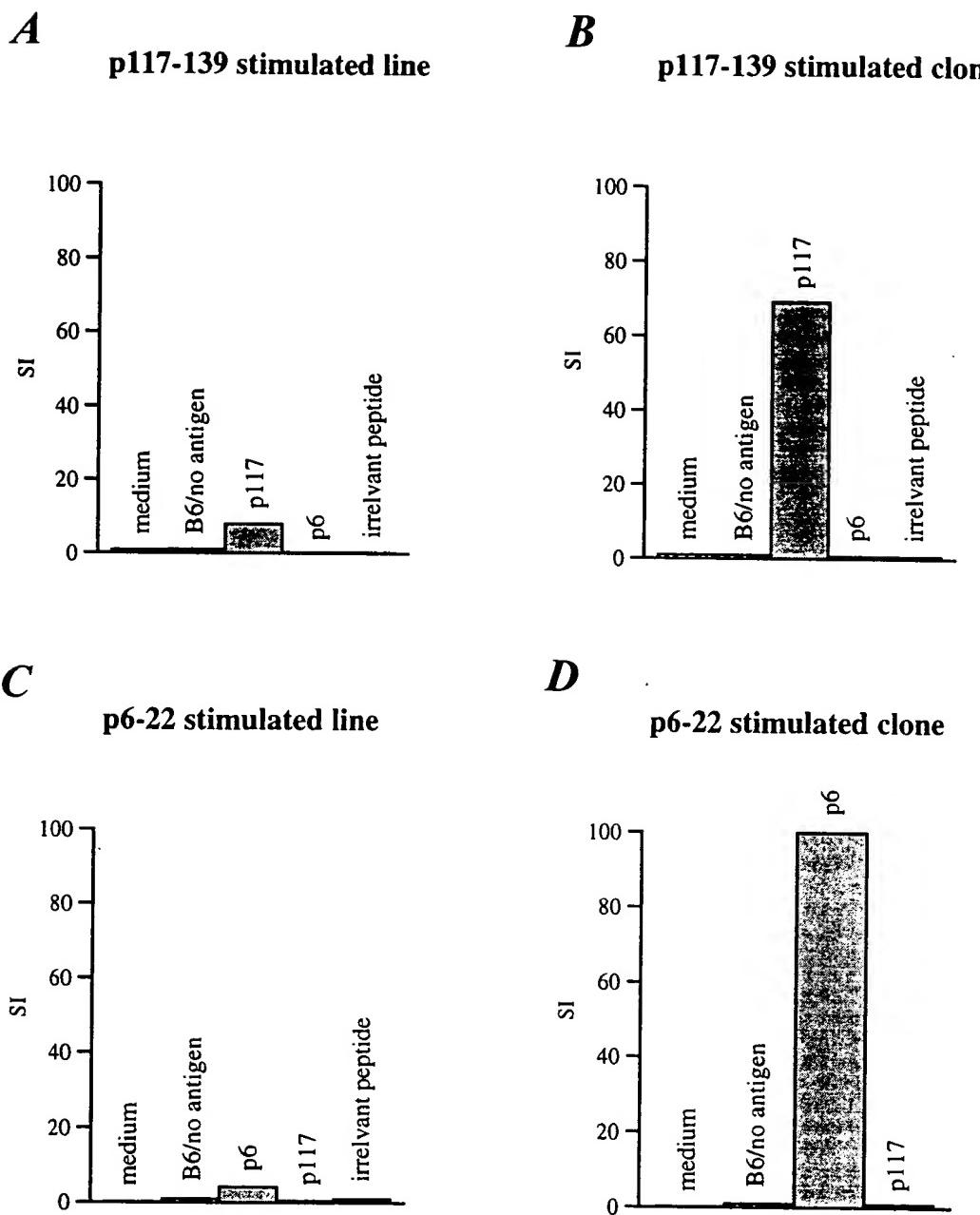
FIG. 7A-7D

```
        5   10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVPSLGGGGGCALPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAAAA.......AAAAAA........AAAAAAAAAAAA.....................
.............................RRRR..........................................
...........................................................................
...........................................................................

       80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTVHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPAIRNQGYS
...............AAA......AAAA.......................AAA.....AAAAAA...........
..............................RRRR.................RRRRR...................
.................................DDDDDDDDD.................................
...........................................................................

      155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGTPSYGHTPSHHAAQFPNHSFKHEDPMGQCGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
..................AAAAA...........................AAAAAA.................AA
..................RRRR....................................................
..........................................................DDDDDDDDDDDDDDD...
...........................................................................

      230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGVAAGSSSSVKWTEGQSNHSTGYESDNHTTPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA...........AAA.AAA...............................AAAAAAAAAAA
..................RRRRRRRRRR.....RRRR.............................RRRR.....
DDDDDD..............DDDDDDDDDD.............................................
...........................................................ddddd.........

      305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVPGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAAA...........................................AAAA.AAAAAAAAA.
....RRRRR.....................RRRR........................................
.......DDDDDD.............................................................
...........................................................................

      380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWPSCQKKFARSDELVRHHNMHQRNMTKLQLAL
.............AAAA.AAAA..AA.....AAAA.........AAA....AAAAAAAA...AAA........
..........................................RRRR..RRRR...............
...........................................................................
.................ddddddddddddd.............................................
```

FIG. 8A

```
          5    10   15   20   25   30   35   40   45   50   55   60   65   70   75
MGSDVRDLNALLPAVSSLGGGGGCGLPVSGAAQWAPVLDFAPPGASAYGSLGGPAPPPAPPPPPPPPPPHSFIKQE
.....AAAAAAAAAAAAAAAAAAA.......AAAAAA........AAAAAAAAAAAA.....................
.................................RRRR.......................................
............................................................................
............................................................................
............................................................................

          80   85   90   95  100  105  110  115  120  125  130  135  140  145  150
PSWGGAEPHEEQCLSAFTLHFSGQFTGTAGACRYGPFGPPPPSQASSGQARMFPNAPYLPSCLESQPTIRNQGYS
........................AAAA...:.................AAA......AAAAAA...........
........................RRRR.....................RRRRR....................
.................................DDDDDDDDD................................
............................................................................

         155  160  165  170  175  180  185  190  195  200  205  210  215  220  225
TVTFDGAPSYGHTPSHHAAQFPNHSFKHEDPMGCCGSLGEQQYSVPPPVYGCHTPTDSCTGSQALLLRTPYSSDN
.............AAAAA...................................AAAAAA.............AA
............RRRR..........................................DDDDDDDDDDDDDDDD...
............................................................................

         230  235  240  245  250  255  260  265  270  275  280  285  290  295  300
LYQMTSQLECMTWNQMNLGATLKGMAAGSSSSVKWTEGQSNHGIGYESDNHTAPILCGAQYRIHTHGVFRGIQDV
AAAAAAAA...........AAA.AAA...............................AAAAAAAAAAAA
.................RRRRRRRRRR....RRRR........................RRRR.....
DDDDDD.............DDDDDDDDDDD...........................................
.....................................................................ddddd........

         305  310  315  320  325  330  335  340  345  350  355  360  365  370  375
RRVSGVAPTLVRSASETSEKRPFMCAYPGCNKRYFKLSHLQMHSRKHTGEKPYQCDFKDCERRFSRSDQLKRHQR
AAAAA..AAAAAAAAAA...............................................AAAA.AAAAAAAAA.
....RRRRR...................RRRR........................................
..DDDDDDDDDD................................................................
............................................................................

         380  385  390  395  400  405  410  415  420  425  430  435  440  445  450
RHTGVKPFQCKTCQRKFSRSDHLKTHTRTHTGKTSEKPFSCRWHSCQKKFARSDELVRHHNMHQRNMTKLHVAL
.............AAAA.AAAA..AA.....AAAA..............AA....AAAAAAAA...AAAA.......
........................................................RRRR..RRRR..............
............................................................................
..............dddddddddddd..................................................
```
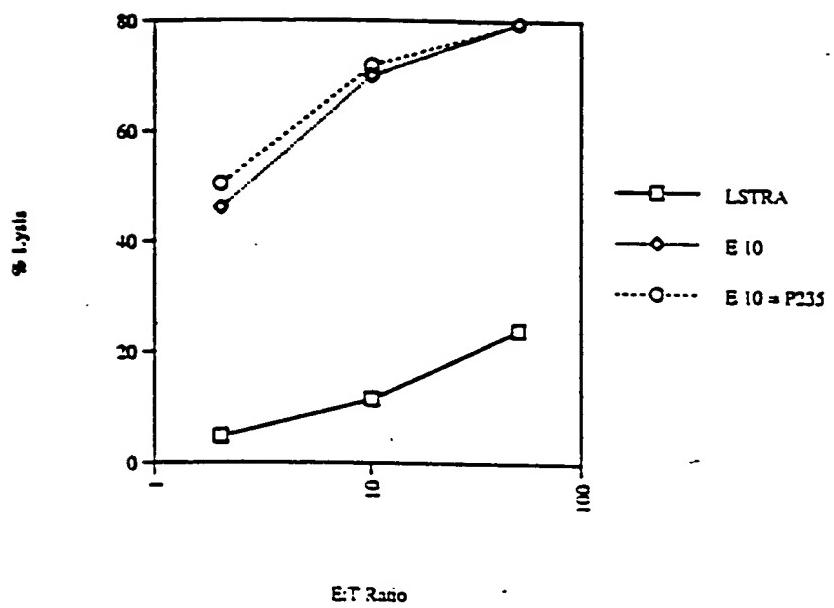
FIG. 8B
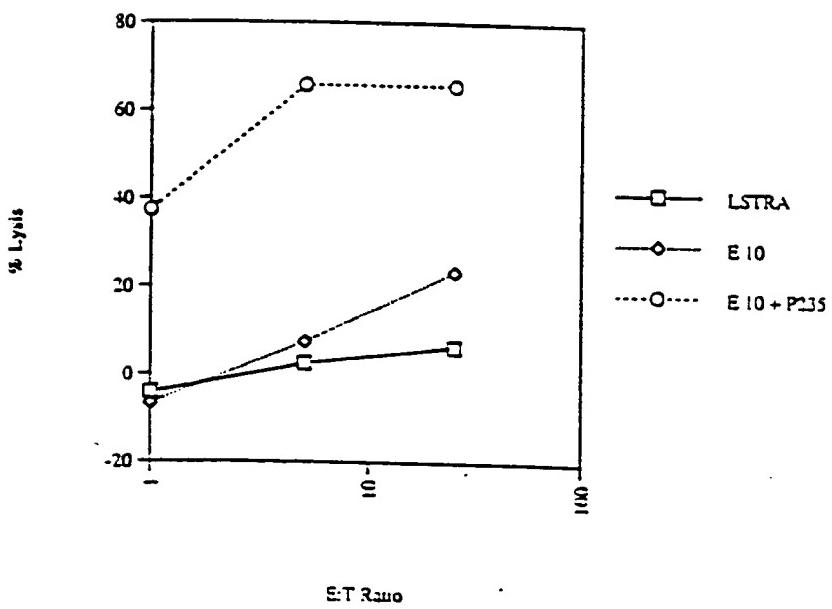
FIG. 9A and 9B
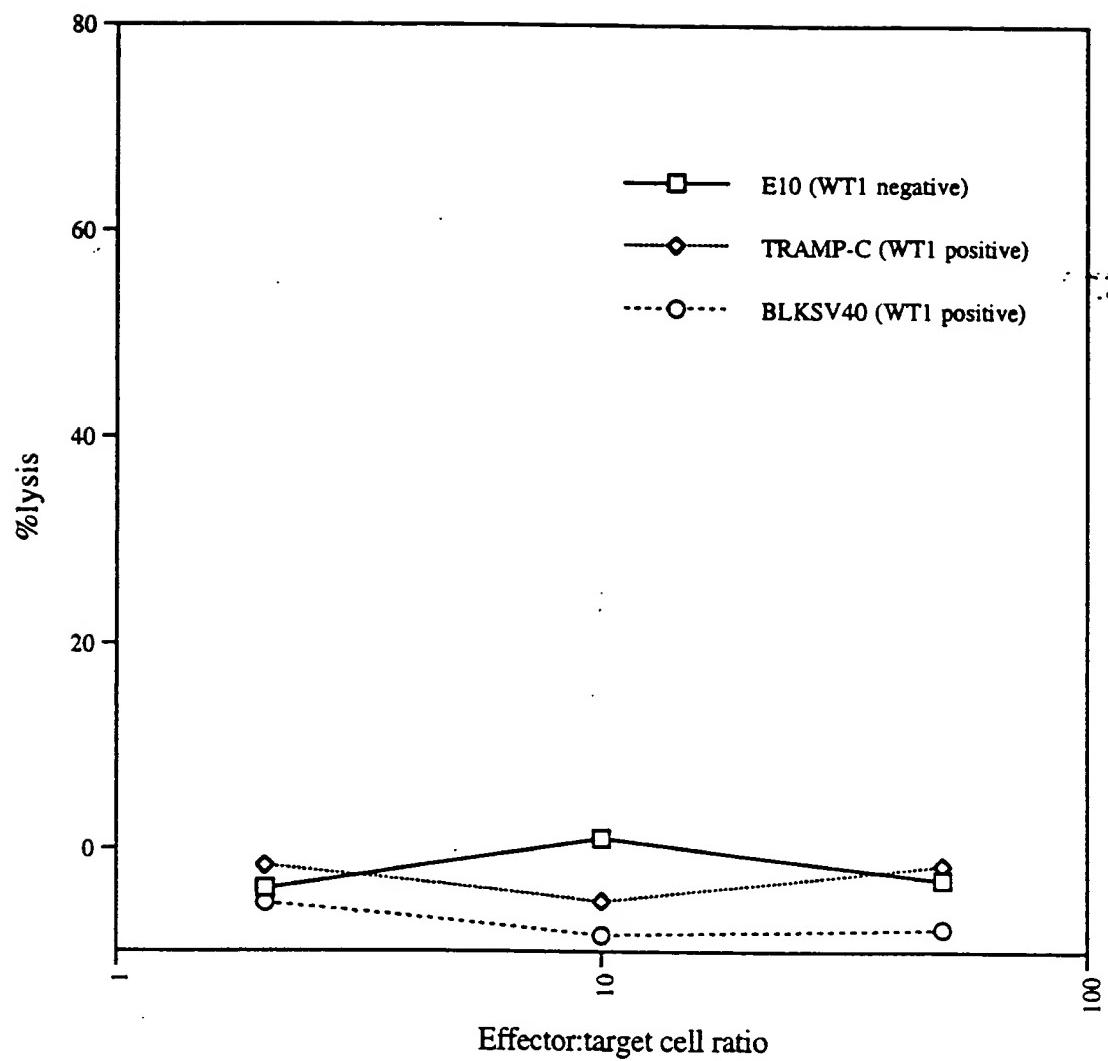
FIG. 10A
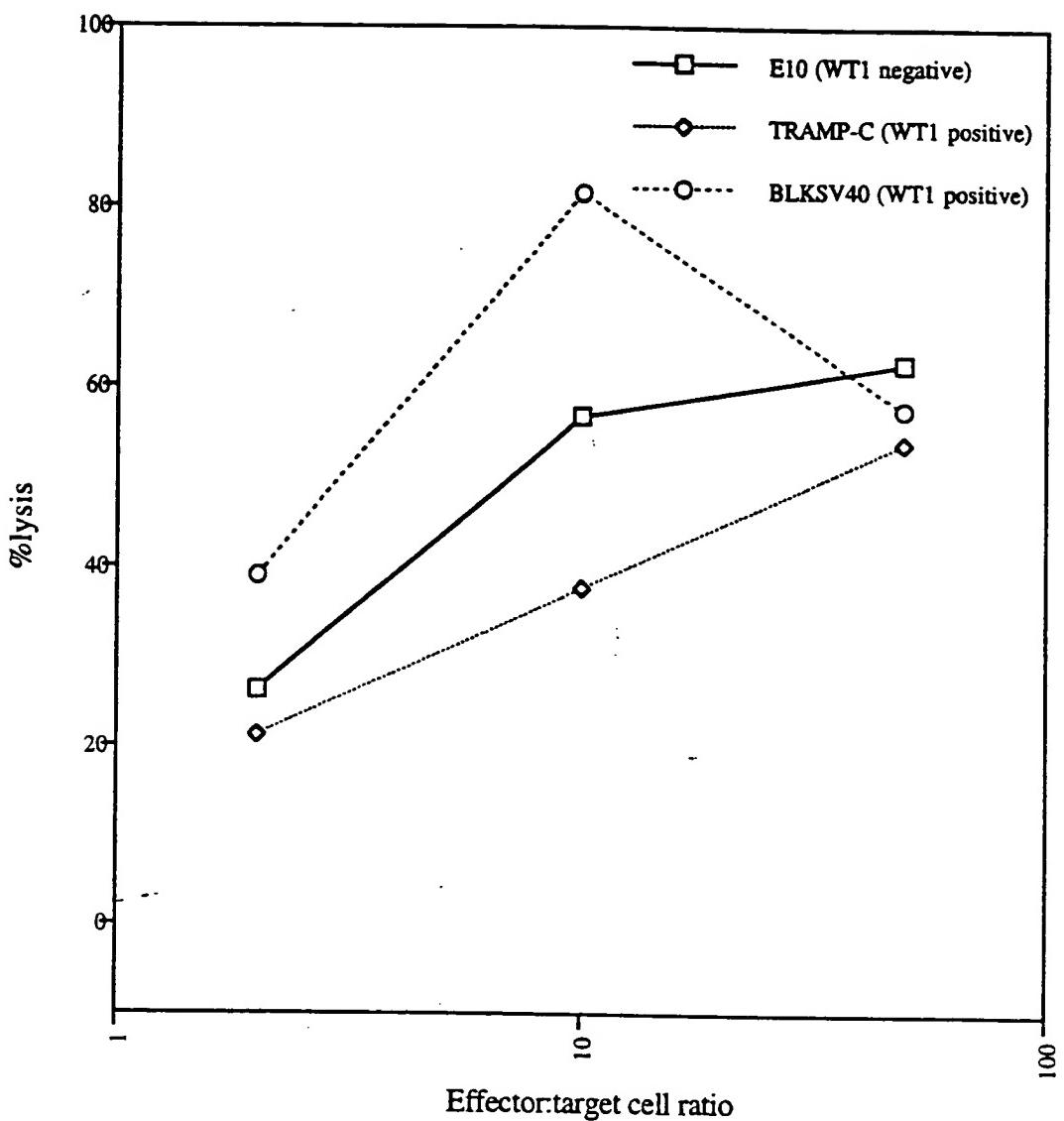
FIG. 10B
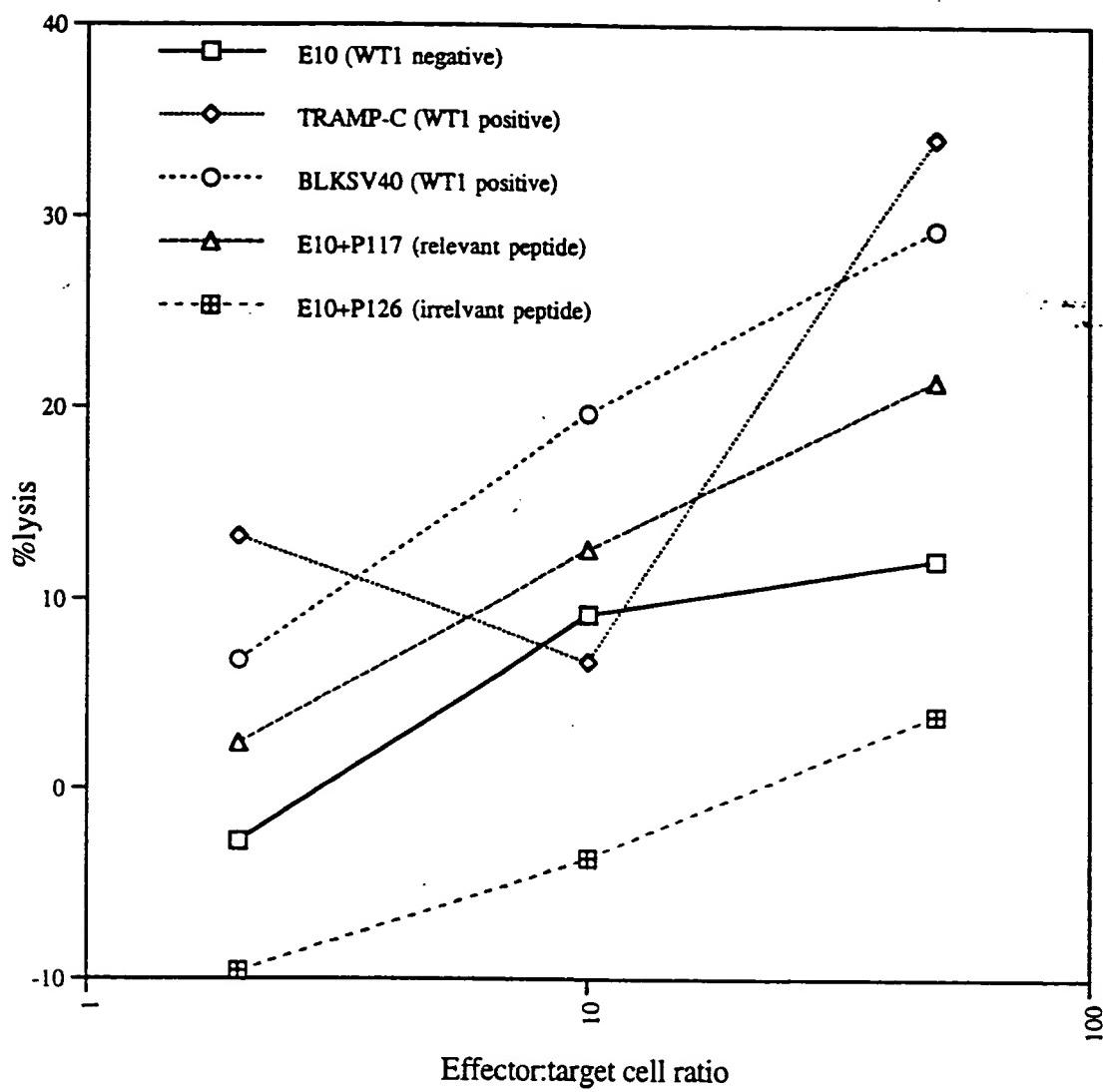
FIG. 10C
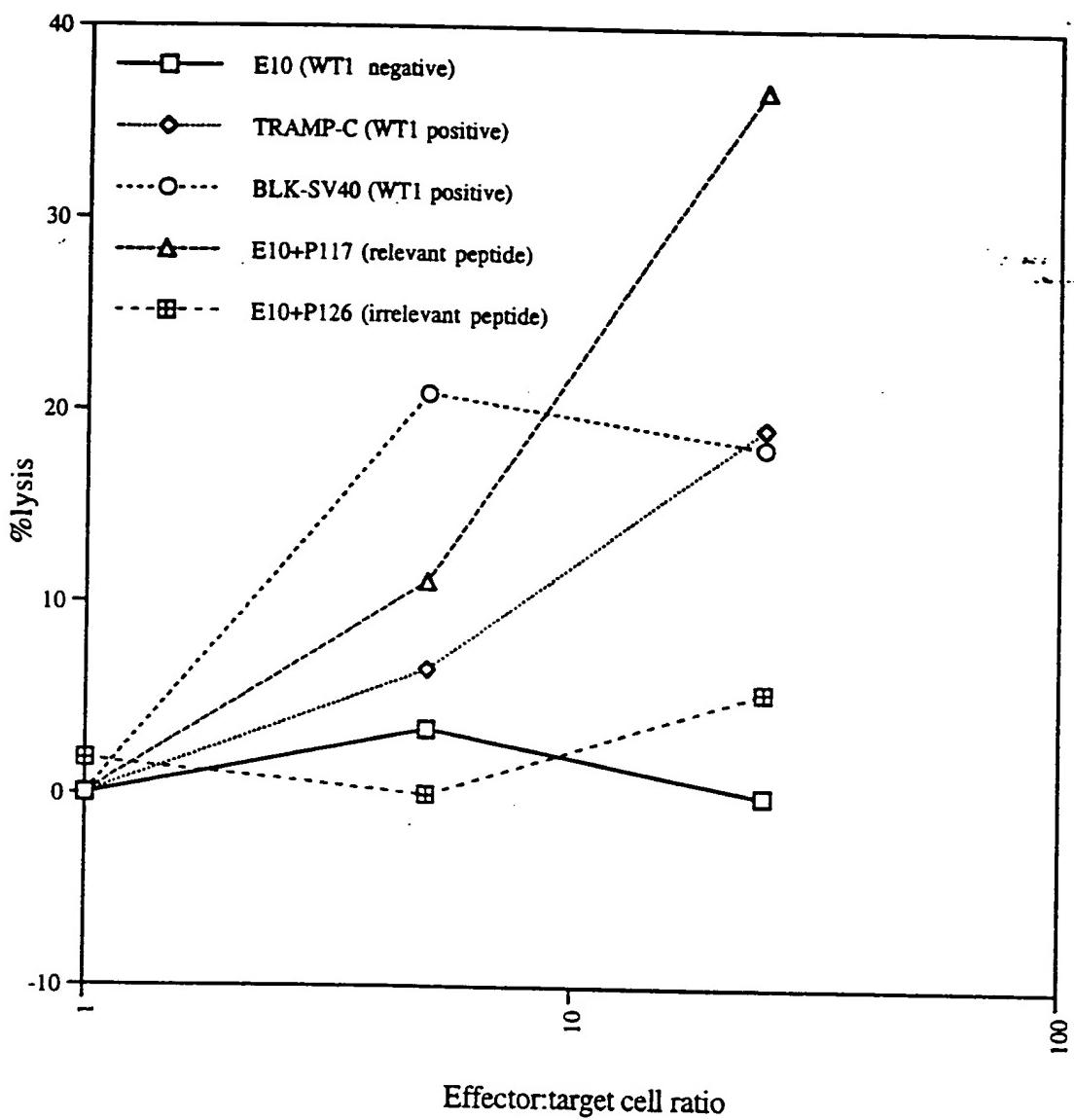
FIG. 10D
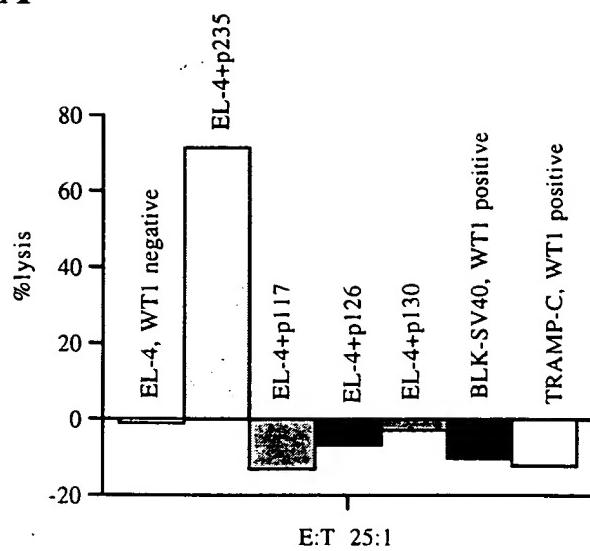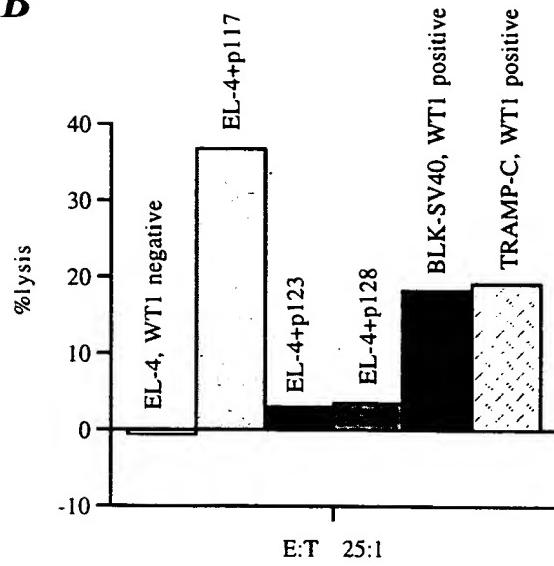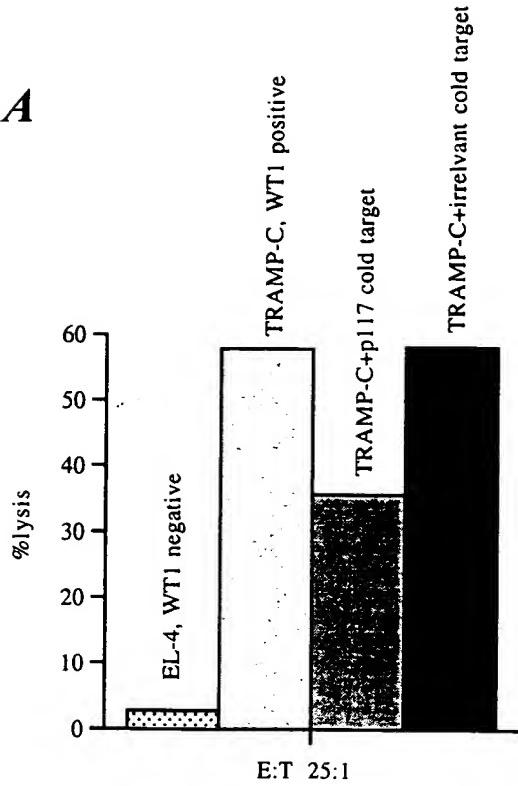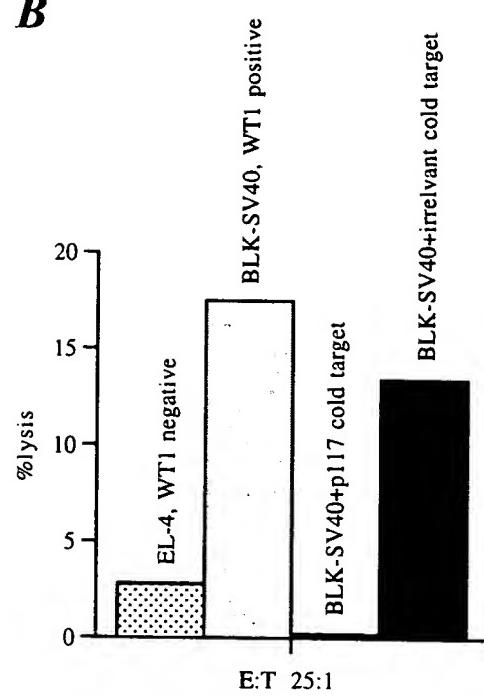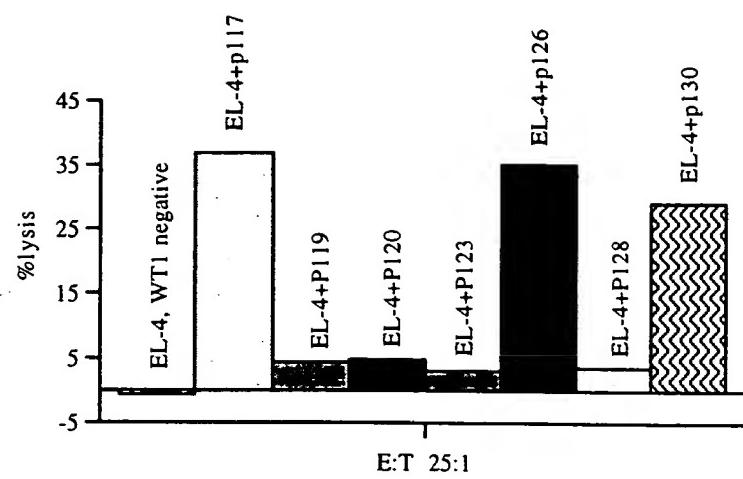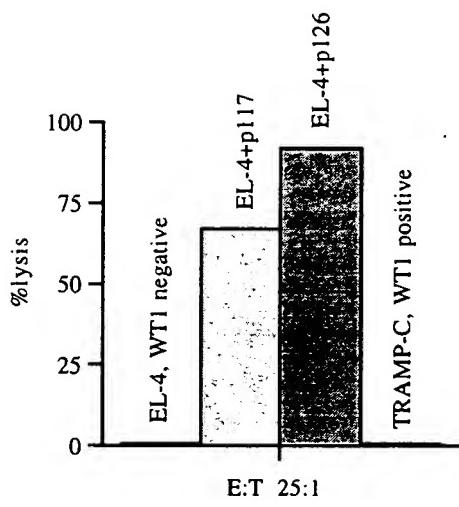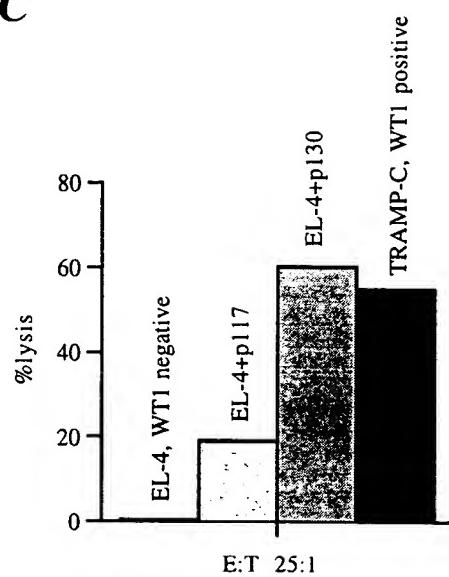
FIG. 11A and 11B

FIG. 12A and 12B

A

%lysis — y-axis: -5, 5, 15, 25, 35, 45

Bars: EL-4, WT1 negative · EL-4+p117 · EL-4+P119 · EL-4+P120 · EL-4+P123 · EL-4+p126 · EL-4+P128 · EL-4+p130

E:T 25:1

B

%lysis — y-axis: 0, 25, 50, 75, 100

Bars: EL-4, WT1 negative · EL-4+p117 · EL-4+p126 · TRAMP-C, WT1 positive

E:T 25:1

C

%lysis — y-axis: 0, 20, 40, 60, 80

Bars: EL-4, WT1 negative · EL-4+p117 · EL-4+p130 · TRAMP-C, WT1 positive

E:T 25:1

FIG. 13A-13C

114
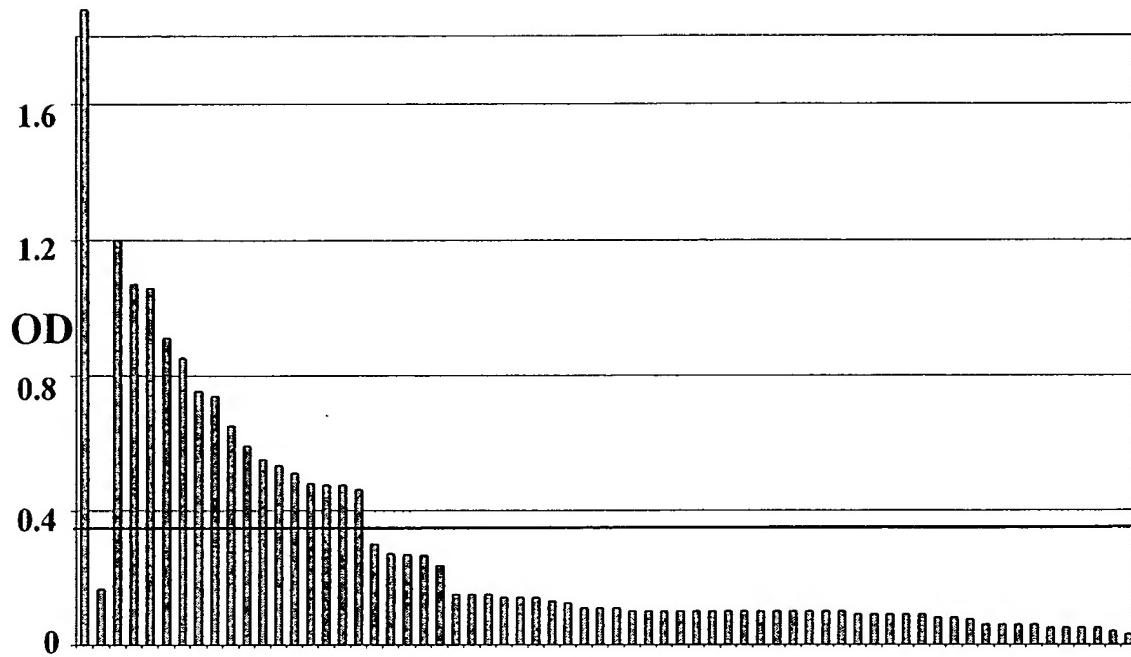
Fig. 14
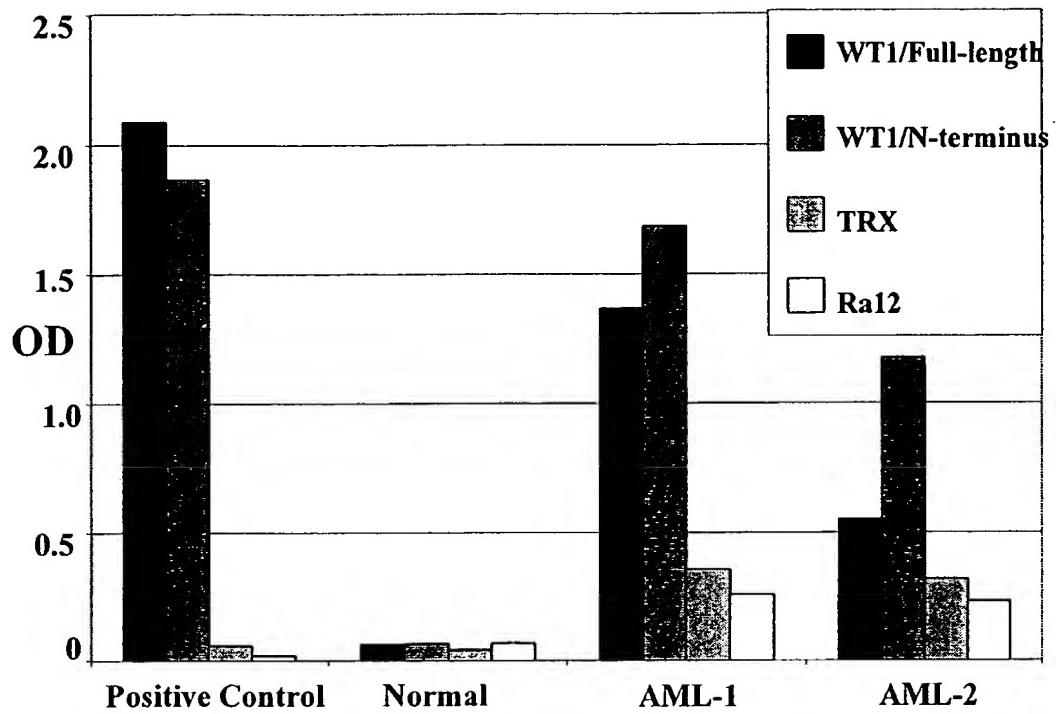
Fig. 15
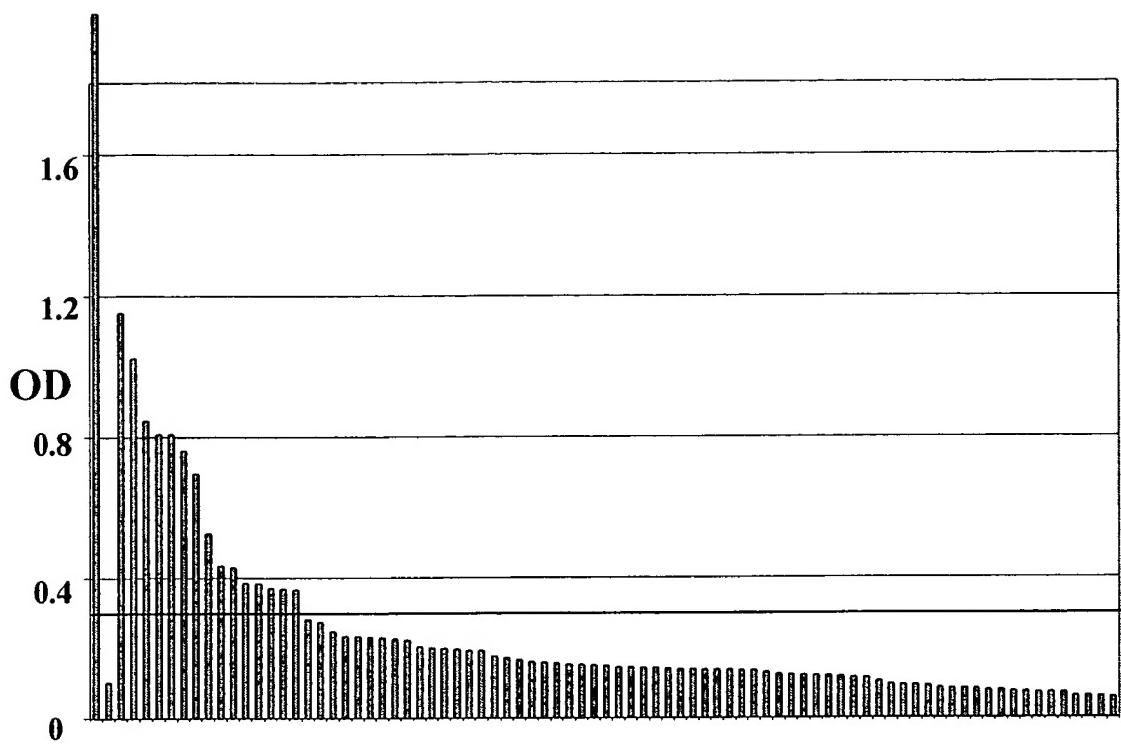
Fig. 16
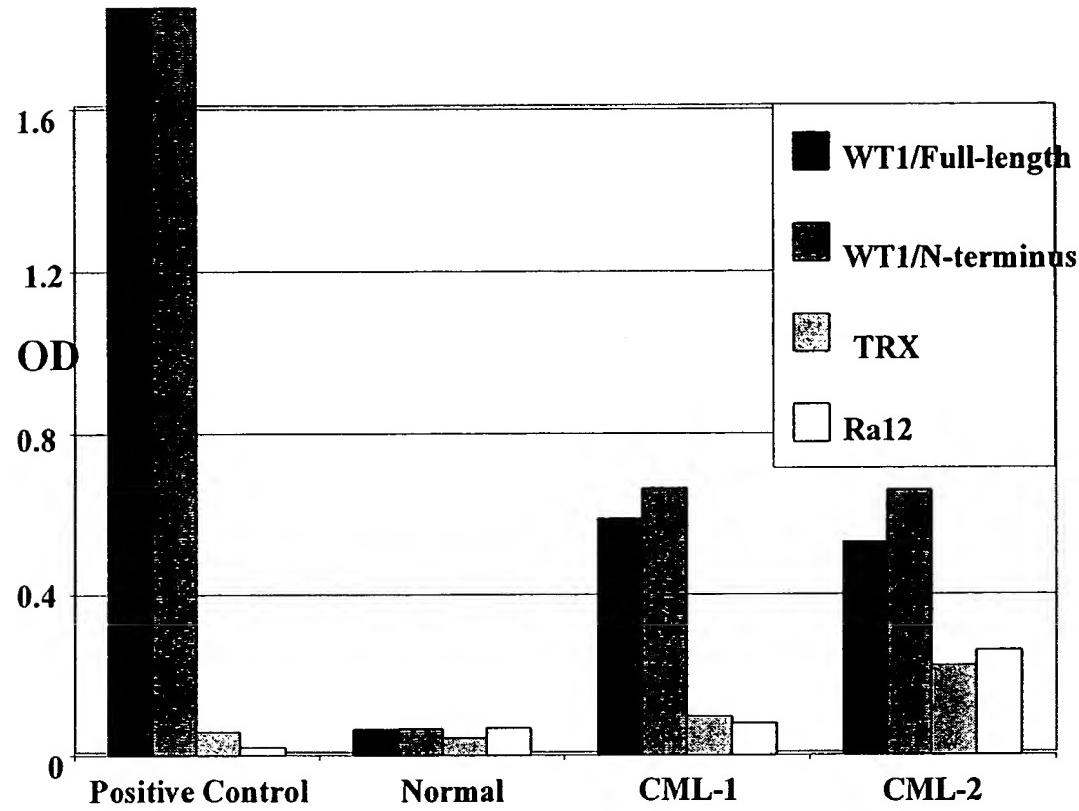
Fig. 17

TABLE 1: Characteristics of Recombinant WT1 Proteins Used for Serological Analysis

Name	Recombinant Protein	WT1 Amino Acid Position	Molecular Weight
WT1/full-length	Ra12-WT1 full length fusion protein	aa 1-449	85kDa
WT1/N-terminus	TRX-WT1 N-terminus fusion protein	aa 1-249	60kDa
WT1/C-terminus	WT1 C-terminus protein	aa 267-449	50kDa

Fig. 18

TABLE 2: WT1 Specific Serum Antibodies in Patients with AML and CML.

	WT1/full-length	WT1/N-terminus	WT1/C-terminus
Normal Individuals (n=96)	2/96 (2%)	1/96 (1%)	1/96 (1%)
AML Patients (n=63)	14/63 (22%)	16/63 (25%)	2/63 (3%)
CML Patients (n=81)	15/81 (19%)	12/81 (15%)	3/81 (3%)

Fig. 19